THE VARIATIONS IN REACTION OF THE BLOOD OF DIFFERENT SPECIES AS INDICATED BY HEMOLYSIS OF THE RED BLOOD CELLS WHEN TREATED WITH ACIDS OR ALKALIES *

JAMES GORDON CUMMING

From the Department of Preventive Medicine of Harvard Medical School, Boston, Mass.

In 1913, at the Pasteur Institute of the University of Michigan an attempt was made to establish the Wassermann reaction on a chemical basis. The research in this direction dealt chiefly with the influence of salts, acids, and alkalies on the hemolytic system. It was first noted that any one of these, even in high dilution, had its influence on this biologic reaction. A specimen known to be negative to the Wassermann test, could be made to give a positive reaction by the addition of a trace of either acid or alkali. Furthermore, on the addition of a somewhat larger quantity of acid or of alkali, a positive specimen would give a negative reaction. Similarly, neutral salts would influence a negative specimen to the extent of causing a positive reaction. The opposite of this, however, was not true.

Further experiments developed the fact that a series of positive and negative reactions, analogous in result to positive and negative Wassermann reactions, could be produced by an acid solution as antigen, and by rabbit serum, rendered acid or alkaline, as positive or negative serum. The acid serum would give a positive end result; while the alkaline serum would be negative. The analogy between the final results of these experiments and those of the Wassermann, coupled with the fact that the actual Wassermann could be influenced with acids or alkalies, and a negative specimen influenced with neutral salts, led to the assumption that the positive Wassermanns might be due to an increase in ions during the first incubation, either acid or alkaline ions, or those of neutral salts.

This assumed to be a fact, the next step in the work was an investigation of the action of acids and alkalies on the hemolysis of certain blood cells. By allowing a fixed time interval for complete hemolysis of a definite blood cell suspension, the percentage of acid or of alkali

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necessary to produce this result could be determined, and this determination could remain fixed for the species thus tested. If a positive Wassermann were due to an increase in ions, either acid or alkali, during the first incubation, the acid or alkaline hemolysis of the second incubation could be correspondingly influenced by either a retardation or an acceleration of the reaction. Should the increased ions be of an alkaline nature, and an acid be used as a hemolytic agent, the time interval would be prolonged; or, if the hemolysin should be alkaline, the interval would be shorter than that of the control. On the other hand, if the increased ions were acid and the hemolytic agents were used in the same order, the results would be reversed. That is, the acid ions produced during the first incubation, coupled with those added as the hemolytic agent, would hasten the reaction, and would retard alkaline hemolysis. At this time the investigation of the hemolytic action of acids and alkalies was limited to the blood cells of 4 species: man, goat, dog, and horse. There was a slight, but well-defined, difference between the blood suspension from man and that from the goat, and between that from the horse and that from the dog. The difference, however, between the blood suspensions of the first group and those of the second was extremely marked,—relatively large quantities of chemical hemolysin being required for the first group, only traces for the second.

Further research in the acid, and alkaline, and salt hemolysis of the blood of the different species was taken up in October, 1914, in the Department of Preventive Medicine at the Harvard Medical School. The results of this work are here presented.

PLAN OF THE WORK

The plan of this research was, first, to determine the percentage of ammonia, sodium hydroxid, hydrochloric acid, and other inorganic and organic compounds, and salts, necessary to cause complete hemolysis of 1% blood-cell suspensions of different species in 15 minutes; second, to determine a chemical index from an arbitrary time index for each of these species; third, to apply these established indices as a means of identifying blood from different species; fourth, to compare the index of the normal with that of the pathologic blood specimen.

The scope of the work as presented in this paper was to establish a chemical standardization for definite hemolytic time indices of dif-

ferent species. This work is to be considered simply preliminary; the chemical indices established by this work being used as a basis, however, it is hoped that by further research our knowledge of the physico-chemical variation in normal and pathologic blood may be extended. Moreover, may not further work along the lines herein reported be applied to a study of living and dead blood, especially as to the ionic content, and the relation of this to the formation of fibrin?

TECHNIC

The blood used in these experiments was obtained by several methods, either by severing the jugular and carotid, or by drawing the blood into a syringe directly from the heart. (The blood should be drawn into a clean container, or, if the syringe be used, this should be rinsed with physiologic salt solution, and the piston be drawn back and driven forcibly in order to expel the last drop of the liquid.) During anesthesia rabbits, guinea-pigs, dogs, monkeys, etc., may be bled from the heart without endangering the life of the animal. Blood from the severed neck vessels of the sheep, ox, chicken, and hog was obtained at the abattoir. In obtaining blood from the frog, rat, and turtle, the animal was first anesthetized; then after exposure of the heart, this was pierced with a pipet, and the blood drawn and finally transferred to a clean container. Blood from man was drawn from the median cephalic vein in the usual manner.

Unless otherwise stated, all blood specimens were defibrinated by gentle whipping with a clean glass rod. The defibrinated blood used in these experiments was in the proportion of 1 c.c. to 99 c.c. of 0.9 % NaCl.

The procedure used in carrying out the various tests was as uniform as possible. The tubes (7 by 80 mm.), which of course were clean, immediately before using were rinsed with 0.9% NaCl solution and drained. They were then placed in the rack, which provides for 12 pairs of tubes. The chemical hemolysin was next measured into the tubes from a double-right-angled pipet. This pipet was standardized to 50 drops of water to the cubic centimeter. It was essential that the drops should be of uniform size, and this uniformity was established by the use of the double-right-angled pipet. To measure accurately 50 drops to the cubic centimeter the dropper arm of the pipet had to be in a perpendicular position; otherwise the size of the drop was increased. Of course any number of drops per cubic centimeter might have been adopted as a standard, but it was essential that these should be uniform and that a standard should be adopted for the entire series of experiments.

Then by adding 1 c.c. of the blood cell suspension to each tube in the series, the immediate mixing of the blood cell suspension with the chemical already in the tube was facilitated somewhat; each pair of tubes was then gently shaken so as to complete the mixing of their contents. Finally the rack was placed in a water-bath at 38 C. (These racks were so constructed that the tubes were immersed to a depth of 2 c.c. with the result that the contents of the tubes were beneath the surface of the water in the bath.) The time of complete hemolysis in each tube in the series was recorded. This series of recorded results represented the time indices, and the equivalent chemical percentages of these indices could then be determined.

The hemolytic time indices of a series of chemical percentages for a given species could be determined only by trial. If, for example, from the standard

pipet from 1 to 12 drops of a definite normality of acid or alkali were placed in a series of 12 tubes and to each of these was added 1 c.c. of a given blood suspension, the time required for complete hemolysis for each percentage of acid or alkali would represent the time index. To illustrate, the hemolysis might be complete in all tubes in a few minutes; or the reaction might be complete in Tube 12 in 5 minutes, and in Tube 1 in 50 minutes. On the other hand, to obtain a given time index, especially a definite time for a certain tube in the series, the experiment became one of considerable difficulty. Moreover, with certain species numerous trials were necessary before the standard time index was determined. This was true particularly with the goat-, dog-, and horse-blood suspensions, each of these requiring many trials.

The series of experiments reported in this paper dealt with the hemolytic effect of the following chemical solutions: ammonia, sodium and potassium hydrate, hydrochloric and sulfuric acid, and acetic acid. These were made up accurately on a normality basis in 0.9% NaCl solution, and, as will be shown later, a sufficient amount of NaCl was added to the standard acid or alkali to render the final solution exactly 0.9% NaCl. To illustrate, 200 c.c. of commercial ammonia were added to 900 c.c. of 0.9% NaCl solution, then 1.8 grams NaCl were added to this solution in order to bring the total NaCl content up to 0.9%. Finally this was titrated, and a sufficient amount of 0.9% NaCl was added to render the ammonia content 3N. NH_3. This salt content, of course, decreased the acid and alkaline content to a lower point than it would have been in a water solution. Nevertheless, this procedure should be taken as a standard method in this work in order to maintain a uniform salt content in all subsequent dilutions with blood cell suspensions. The chemical indices as well as other chemical considerations are given on a percentage basis, and the tables applied for transposing the standard solutions to their equivalent percentages are here presented.

Should the acid or alkaline solution be made up in water, it is obvious that, if unequal quantities were added to uniform quantities of blood cell suspensions in 0.9% NaCl solution, the salt content would vary. For instance, if one drop of an ammonia solution in water be added to 1 c.c. of a blood cell suspension in 0.9% NaCl solution, the final NaCl content, according to the formula $51 : 50 :: 0.9 : X$, would be 0.88% NaCl. If diluted with 12 drops, it would be, $62 : 50 :: 0.9 : X$, or 0.72% NaCl. These variations in the final salt content have a definite effect on the period of hemolysis. If, on the other hand, the acids and alkalies be in 0.9% NaCl solution, the addition of these to the blood cell suspensions will be accompanied by no decrease in the standard salt percentage.

To ascertain the time index of a certain blood cell suspension the procedure in these experiments was as follows: First, in a series of 12 pairs of tubes 1 drop of the chemical hemolysin was placed in one tube of the first pair (its mate serving as a control), 2 drops in the corresponding tube of the second pair, and so on successively, including the tube of the twelfth pair, which received 12 drops. Second, 1 c.c. of the blood cell suspension was then run into each tube in the series. Third, each pair of tubes was removed from the rack, turned at an angle of 45, and shaken. Fourth, the rack was then placed in

TABLE 1

TABLE APPLIED IN THIS WORK FOR TRANSPOSING STANDARD SOLUTIONS OF NH_3 TO THEIR CORRESPONDING PERCENTAGES

		3N	2N	N/1	N/2	N/3	N/4	N/5	N/10	N/15	N/60
1	N/51.....	.1	.066	.033	.016	.011	.0083	.0066	.0033	.0022	.0005
2	N/26.....	.196	.13	.0654	.0327	.0218	.0162	.013	.0065	.0043	.001
3	N/17.6.....	.288	.192	.0964	.0432	.0321	.0241	.0193	.0096	.0064	.0016
4	N/13.5.....	.378	.252	.126	.063	.042	.031	.025	.0126	.0084	.0021
5	N/11.....	.463	.308	.154	.077	.051	.038	.031	.0154	.01	.0025
6	N/9.3.....	.548	.364	.182	.091	.061	.054	.036	.018	.012	.003
7	N/8.2.....	.621	.414	.207	.103	.069	.052	.041	.021	.013	.0034
8	N/7.2.....	.708	.472	.236	.118	.079	.059	.047	.023	.015	.0039
9	N/6.5.....	.783	.522	.261	.130	.087	.065	.052	.026	.017	.0043
10	N/6..,.....	.849	.566	283	.141	.094	.071	.056	.028	.018	.0047
11	N/5.5.....	.927	.618	.308	.154	.103	.077	.062	.031	.02	.0051
12	N/5.16.....	.978	.652	.326	.163	.109	.081	.065	.034	.022	.0054

TABLE 2

TABLE APPLIED IN THIS WORK FOR TRANSPOSING NaOH MOLECULAR SOLUTIONS TO THEIR CORRESPONDING PERCENTAGES

		N/1	N/10	N/12	N/15	N/25	N/28	N/30	N/35	N/50	N/90
1	N/51.....	.0784	.0078	.0065	.0052	.0031	.0028	.0026	.0022	.0015	.0009
2	N/26.....	.153	.0153	.012	.01	.0061	.0055	.0051	.0043	.003	.0017
3	N/17.6.....	.227	.0227	.018	.015	.0093	.0081	.0075	.0064	.0043	.0025
4	N/13.5.....	.2962	.0296	.024	.019	.0118	.0106	.0098	.0084	.0058	.0032
5	N/11.....	.3636	.0363	.03	.024	.0145	.013	.012	.0104	.0072	.004
6	N/9.3.....	.43	.042	.035	.028	.0172	.0155	.0143	.0122	.0086	.0048
7	N/8.2.....	.488	0488	.04	.032	.019	.0173	.016	.0139	.0096	.0054
8	N/7.2.....	.555	.0555	.046	.037	.022	.0198	.018	.0157	.011	.0061
9	N/6.5.....	.615	.0615	.051	.041	.024	.0221	.0205	.0175	.0122	.0068
10	N/6.....	.666	.0666	.055	.044	.026	.024	.0222	.019	.0132	.0074
11	N/5.5.....	.727	.0727	.06	.048	.029	.0261	.0242	.0208	.0144	.008
12	N/5.16.....	.775	.0775	.064	.052	.031	.028	.0258	.0221	.0154	.0086

TABLE 3

TABLE APPLIED IN THIS WORK FOR TRANSPOSING HCl MOLECULAR SOLUTIONS TO THEIR CORRESPONDING PERCENTAGES

		N/50	N/75	N/80	N/100	N/120	N/150
1	N/51.....	.0014	.0009	.0008	.0007	.0006	.0004
2	N/26.....	.0028	.0018	.0017	.0014	.0011	.0009
3	N/17.6.....	.0041	.0027	.0025	.002	.0017	.0015
4	N/13.5.....	.0054	.0035	.0033	.0027	.0022	.0017
5	N/11.....	.0066	.0043	.0041	.0033	.0027	.0021
6	N/9.3.....	.0079	.0052	.0048	.0039	.0033	.0026
7	N/8.2.....	.0089	.0058	.0055	.0044	.0037	.0029
8	N/7.2.....	.01	.0066	.0062	.005	.0042	.0033
9	N/6.5.....	.0112	.0072	.0068	.0055	.0046	.0036
10	N/6.....	.012	.0079	.0074	.006	.005	.0039
11	N/5.5.....	.013	.0085	.0081	.0065	.0054	.0042
12	N/5.16.....	.014	.0092	.0086	.007	.0058	.0046

EXPLANATION OF TABLES 1, 2, AND 3

The first vertical row represents the serial tubes in the rack, also the number of drops of the standard solution added: 1 drop to the first tube, 2 drops to the second tube, etc., to 12 drops to the twelfth tube. The second row states the normality coinciding with the number of drops added to an N solution. The following successive rows represent the percentages of the chemical corresponding to the normality indicated at the top of each column, and to the number of drops in the series.

the water-bath, and finally a record made of the time of the completion of hemolysis in each tube.

As the rapidity of hemolysis depends on the percentage of the hemolytic agent, it was found that, with the exception of hydrochloric acid, Tube 12, i. e., the one containing the highest percentage, hemolyzed first in the series and Tube 1 last.

If preliminary tests be made on a given species, using as the hemolytic agent percentages of a certain chemical, the time indices for the series of tubes may be comparatively short—1 minute in the twelfth tube, and 10 minutes in the first tube. As has been stated, the first problem was to determine the chemical percentages necessary to complete hemolysis in 15 minutes. Furthermore, it was found, as the work progressed, that the fifteen-minute hemolytic system should occur in the fifth tube in the series. This is advisable in order that the factor of dilution of the blood components—acid, alkaline, and neutral salt ions, serum, and cells—with the hemolytic agent be kept as nearly uniform as possible for the fifteen-minute hemolytic system. If the fifteen-minute hemolytic system be in the fifth tube for one test, and in the twelfth tube for another, the variation in the percentages of blood components would be 0.909% in the fifth tube, and 0.806% in the twelfth tube; this variation in dilution would cause an appreciable lack of uniformity in results.

As in the test described, if all tubes are hemolyzed in 10 minutes, it is obvious that the chemical hemolytic agent should be reduced. Inasmuch as the number of drops remains the same in each set of tubes, it is necessary to reduce the percentage or normality of the standard chemical solution. As no exact rule as yet appears applicable to all species for the reduction of percentage or normality, that normality which produces complete hemolysis in the fifth tube in the series in 15 minutes can be ascertained only by trial. After having found the normality which will give hemolysis in the fifth tube in 15 minutes, the chemical percentages can be determined for each tube in the series, and the recorded results serve as the hemolytic time indices.

It was necessary to find whether or not these percentages would give the same time indices for numerous specimens, each taken from a different animal of the same species. (Unless otherwise stated, the blood cell suspensions were from normal animals.)

That this work at the present stage of its progress is subject to minor errors, must be taken into consideration. These, however, may be eliminated as the technical details are perfected. Small quantities of both chemicals and blood cell suspensions were used, and the cell suspension was only 1%. The difficulty of accurately measuring 1 c.c. from a 10 c.c. pipet accounts, at least to some extent, for the slight variation in the time indices for the same species and the same chemical percentages. This is particularly true of those high chemical dilutions in the first tubes of the series; while it is not of great importance with the lower dilutions. Should the amount of cell suspension be increased to several cubic centimeters with a corresponding increase in chemical, this alone would tend to give more accurate results. Furthermore, should the percentage of cell suspension be increased from 1%—that adopted in this work—to 5 or 10%, it is obvious that with the same time indices, there would be a relatively greater variation in the chemical percentages. Primarily, the object in using a 1% cell suspension was that, if with this low percentage, a difference in species could be shown, then, with the higher percentages, there could be no question concerning this point.

The difference between the chemical percentages for the same time indices of the horse and the mule was not great for the 1% blood cell suspension, but when the suspension was increased to 10%, the difference at once became more evident (Charts VI and VII). Tho this was true for these two animals, it is not necessarily so for other species, for there is no exact proportional relation between the percentage of the blood cells, the chemical percentage, and the time of complete hemolysis. That is to say, 1% defibrinated blood suspensions may with the same chemical index give different time indices, but should both suspensions be increased to 10% both may give the same time index, or the one giving the shorter time index with the 1% may even give the longer index with the 10% blood suspension. In short, the law of definite proportions applies to this work to a limited extent only. It may be stated, also, that this law does not apply to the chemical and blood cell percentages. A 1% cell suspension gives definite time indices for a fixed series of chemical percentages; when the cell suspension is increased to 10% it is found that less than 10 times the chemical percentage is required to give approximately the same time index. The constituents of the blood serum may be responsible for the irregular proportional relationship, and may it not be assumed

TABLE 4

TABLE OF CHEMICAL EQUIVALENTS IN PERCENTAGE OF A 15-MINUTE HEMOLYTIC SYSTEM FOR 1% BLOOD CELL SUSPENSIONS OF DIFFERENT SPECIMENS FROM THE SAME SPECIES

Animal	Drops	NH_3	Percentage	Drops	NaOH	Percentage	Drops	HCl	Percentage
Goat 1	6	3N	.548	4	N/10	.0296	7	N/120	.00325
2	6	3N	.548	4	N/10	.0296	7	N/120	.00325
3*	5	3N	.463	4	N/10	.0296	7	N/124	.0036
Cow (Bovine) 1	6	2N	.364	4	N/12	.024	3	N/80	.0026
2	6	2N	.364	4	N/12	.024	3	N/80	.0026
3	6	2N	.364	4	N/12	.024	3	N/80	.0026
4	6	2N	.364	4	N/12	.024	3	N/80	.0026
5	4	3N	.378	4	N/12	.024	3	N/80	.0026
6	4	3N	.378	4	N/12	.024	3	N/80	.0026
7	4	3N	.378	4	N/12	.024	2	N/80	.0018
8	4	3N	.378	4	N/12	.024	2	N/80	.0018
9	4	3N	.378	4	N/12	.024	3	N/80	.0026
10	4	3N	.378	4	N/12	.024	3	N/80	.0026
11	4	3N	378	4	N/12	.024	3	N/80	.0026
Sheep 1	7	2N	.414	6	N/15	.028	6	N/90	.0044
2	4	3N	.378	5	N/15	.024	8	N/150	.0033
3	4	3N	.378	5	N/15	.024	10	N/150	.0039
4	4	3N	.378	5	N/15	.024	9	N/150	.0036
5	4	3N	.378	5	N/15	.024	9	N/150	.0036
6	4	3N	.378	5	N/15	.024	6	N/100	.0039
Deer 1	6	2N	.364	6	N/25	.0172	7	N/75	.0058
Negro 1	11	N	.308	7	N/25	.019	7	N/100	.0044
2	11	N	.308	7	N/25	.019	7	N/100	.0044
3	5	2N	.308	6	N/25	.0172	7	N/100	.0044
4	5	2N	.308	6	N/25	.0172	7	N/100	.0044
Elephant 1	5	2N	.308	7	N/25	.019	2	N/75	.0018
Cat 1	3	3N	.288	5	N/15	.024	8	N/100	.005
2	3	3N	.288	5	N/15	.024	8	N/100	.005
3	3	3N	.288	5	N/15	.024	8	N/100	.005
4	3	3N	.288	6	N/15	.028	10	N/100	.006
5	3	3N	.288	5	N/15	.024	8	N/100	.005
6	3	3N	.288	6	N/15	.028	10	N/100	.006
7	3	3N	.288	5	N/15	.024	8	N/100	.005
8	3	3N	.288	4	N/15	.019	9	N/100	.0056
9	4	3N	.378	5	N/15	.024	4	N/100	.0026
10	3	3N	288	4	N/15	.019	5	N/100	.0033
11†	3	3N	.288	4	N/15	.019	5	N/150	.0021
12†	3	3N	.288	4	N/15	.019	5	N/150	.0021
13†	3	3N	.288	4	N/15	.019	5	N/150	.0021
Monkey 1	6	N	.182	7	N/35	.0139	6	N/100	.0039
2	7	N	.207	7	N/35	.0139	5	N/100	.0033
3	7	N	.207	7	N/35	.0139	6	N/100	.0039
4	7	N	.207	7	N/35	.0139	6	N/100	.0039
5	7	N	.207	7	N/35	.0139	5	N/100	.0033
6‡	4	N	.207	4	N/35	.0084	6	N/75	.0052
Caucasian 1	3	2N	.192	7	N/28	.0155	4	N/50	.0054
2	6	N	.182	7	N/28	.0155	4	N/50	.0054
3	6	N	.182	7	N/28	.0155	6	N/75	.0052
4	...	.....	.....	7	N/28	.0155	6	N/75	.0052
5	5	N	.1546	6	N/25	.0172	6	N/80	.0048
6	5	N	.1546	6	N/25	.0172	7	N/80	.0055
7	5	N	.1546	6	N/25	.0172	6	N/80	.0048
8	6	N	.182	6	N/25	.0172	6	N/75	.0052
9	5	N	.1546	5	N/25	.0144	6	N/75	.0052
10	5	N	.1546	6	N/25	.0172	6	N/75	.0052
11	5	N	.1546	6	N/25	0.172	6	N/75	.0052
12	5	N	.1546	5	N/25	.0144	6	N/75	.0052
13	6	N	.182	6	N/25	.0172	9	N/100	.0055
14	6	N	.182	6	N/25	.0172	9	N/100	.0055

* Sensitized to rabbit red blood cells.
† Two-day-old kittens.
‡ Case of cage paralysis.

TABLE 4—*Continued*

TABLE OF CHEMICAL EQUIVALENTS IN PERCENTAGE OF A 15-MINUTE HEMOLYTIC SYSTEM FOR 1% BLOOD CELL SUSPENSIONS OF DIFFERENT SPECIMENS FROM THE SAME SPECIES

Animal	Drops	NH_3	Percentage	Drops	NaOH	Percentage	Drops	HCl	Percentage
Swine 1	6	N	.182	6	N/28	.0155	6	N/75	.0052
2	6	N	.182	6	N/28	.0155	6	N/75	.0052
3	6	N	.182	6	N/28	.0155	6	N/72	.0052
4	6	N	.182	6	N/30	.0143	6	N/75	.0052
5	5	N	.154	6	N/30	.0143	6	N/75	.0052
6	5	N	.154	6	N/30	.0143	6	N/75	.0052
7	5	N	.154	6	N/30	.0143	6	N/75	.0052
8	5	N	.154	6	N/30	.0143	6	N/75	.0052
9	5	N	.154	6	N/30	.0143	6	N/75	.0052
10	5	N	.154	6	N/30	.0143	6	N/75	.0052
Frog 1	4	N	.126	5	N/25	.0145	4	N/75	.0035
2	4	N	.126	5	N/25	.0145	4	N/75	.0035
3	4	N	.126	5	N/25	.0145	4	N/75	.0035
4	4	N	.126	5	N/25	.0145	4	N/75	.0035
Pigeon 1	4	N	.126	7	N/30	.016			
2	4	N	.126	7	N/30	.016			
Chicken 1	6	N/2	.091	6	N/25	.0172			
2	7	N/2	.103	6	N/25	.0172			
3	5	N/1.75	.088	5	N/25	.0145			
Rabbit 1	6	N/2	.091	6	N/35	.0122	4	N/40	.0067
2	5	N/2	.077	6	N/35	.0122	4	N/40	.0067
3	4	N/2	.063	6	N/35	.0122	5	N/50	.0066
4	7	N/3	.069	6	N/35	.0122	5	N/50	.0066
5	7	N/3	.069	6	N/35	.0122	5	N/50	.0066
6	4	N/1.75	.066	6	N/35	.0122	5	N/50	.0066
7	4	N/2	.063	5	N/35	.01	5	N/50	.0066
8	4	N/2	.063	5	N/35	.01	5	N/50	.0066
9§	2	N/1.75	.037	6	N/35	.0122	4	N/50	.0054
10‖	2	N/2	.0327	4	N/35	.0084	8	N/50	.01
Guinea-pig 1	6	N/3	.061	5	N/30	.012	4	N/75	.0035
2	6	N/3	.061	5	N/30	.012	4	N/75	.0035
3	6	N/3	.061	...	.....	.....	4	N/75	.0035
4	6	N/3	.061	...	.....	.....	4	N/75	.0035
5	4	N/2	.0629	7	N/50	.0099	5	N/100	.0033
6	4	N/2	.0629	7	N/50	.0099	5	N/100	.0033
7	4	N/2	.0629	8	N/50	.0104	11	N/200	.0033
8	4	N/2	.0629	8	N/50	.0104	11	N/200	.0033
9	4	N/2	.0629	8	N/50	.0104	11	N/200	.0033
10	4	N/2	.0629	8	N/50	.0104	11	N/200	.0033
11	4	N/2	.0629	8	N/50	.0104	5	N/100	.0033
12	4	N/2	.0629	8	N/50	.0104	5	N/100	.0033
Turtle 1	3	N/2	.043	4	N/35	.0084	3	N/75	.0027
2	3	N/2	.043	3	N/35	.0064	4	N/75	.0035
3	3	N/2	.043	4	N/35	.0084	3	N/75	.0027
4	3	N/2	.043	4	N/35	.0084	3	N/75	.0027
5	3	N/2	.043	4	N/35	.0084	...	.....	.....
6	3	N/2	.043	3	N/35	.0064	...	.....	.....
Brown Rat 1	4	N/3	.042	4	N/25	.0118	4	N/80	.0033
2	4	N/3	.042	4	N/25	.0118	4	N/80	.0033
3	4	N/3	.042	4	N/25	.0118	4	N/80	.0033
4	4	N/3	.042	4	N/25	.0118	4	N/80	.0033
5	4	N/3	.042	4	N/25	.0118	4	N/80	.0033
6	4	N/3	.042	4	N/25	.0118	4	N/80	.0033
7	6	N/4	.045	4	N/35	.0084	5	N/75	.0043
8	6	N/4	.045	4	N/35	.0084	5	N/75	.0043
9	6	N/4	.045	4	N/35	.0084	4	N/75	.0035
10	6	N/4	.045	4	N/35	.0084	4	N/75	.0035
12	6	N/4	.045	4	N/35	.0084	4	N/75	.0035
13	6	N/4	.045	4	N/35	.0084	4	N/75	.0035

§ Severe case of so-called snuffles.
‖ Streptococcus septicemia—see chart.

TABLE 4—*Continued*

TABLE OF CHEMICAL EQUIVALENTS IN PERCENTAGE OF A 15-MINUTE HEMOLYTIC SYSTEM FOR 1% BLOOD CELL SUSPENSIONS OF DIFFERENT SPECIMENS FROM THE SAME SPECIES

Animal	Drops	NH_3	Percentage	Drops	NaOH	Percentage	Drops	HCl	Percentage
White Rat 1	5	N/4	.038	5	N/50	.0072	5	N/75	.0043
2	5	N/4	.038	5	N/50	.0072	5	N/75	.0043
3	5	N/4	.038	5	N/50	.0072	5	N/75	.0043
4	5	N/4	.038	5	N/50	.0072	5	N/75	.0043
Mule 1	6	N/15	.012	4	N/28	.0106	6	N/100	.0039
2	8	N/15	.015	5	N/28	.013	6	N/100	.0039
3	6	N/15	.012	5	N/28	.013	6	N/100	.0039
4	7	N/15	.013	5	N/28	.013	6	N/100	.0039
Horse 1	4	N/15	.0084	5	N/28	.013	5	N/100	.0033
2	5	N/15	.0103	5	N/28	.013	5	N/100	.0033
3	5	N/15	.0103	5	N/28	.013	5	N/100	.0033
4	4	N/15	.0084	5	N/28	.013	5	N/100	.0033
5	5	N/15	.0103	5	N/28	.013	5	N/100	.0033
6	4	N/15	.0084	5	N/28	.013	5	N/100	.0033
Bear 1	5	N/60	.0025	3	N/50	.0045	7	N/100	.0044
Dog 1	5	N/60	.0025	3	N/90	.0025	6	N/100	.0039
2	5	N/60	.0025	3	N/90	.0025	6	N/100	.0039
3	5	N/60	.0025	3	N/90	.0025	6	N/100	.0039
4	4	N/60	.0021	5	N/120	.003			
5	4	N/60	.0021	5	N/90	.004	5	N/75	.0043
6	4	N/60	.0021	5	N/90	.004	5	N/100	.0033
7	4	N/60	.0021	5	N/90	.004	5	N/80	.0041
8	4	N/60	.0021	5	N/90	.004	4	N/80	.0033
9	5	N/60	.0025	3	N/90	.0025	4	N/80	.0033
10	5	N/60	.0025	4	N/90	.0032	4	N/80	.0033
11	4	N/60	.0021	4	N/90	.0032	4	N/80	.0033
12	4	N/60	.0021	3	N/90	.0025	6	N/100	.0039
13	4	N/60	.0021	4	N/90	.0032	6	N/100	.0039
14	5	N/60	.0025	3	N/90	.0025	6	N/100	.0039
15	4	N/60	.0021	3	N/90	.0025	7	N/100	.0044
16	3	N/90	.0011	4	N/90	.0032	9	N/120	.0046
17	3	N/90	.0011	4	N/90	.0032	9	N/120	.0046
18	3	N/90	.0011	4	N/90	.0032	8	N/120	.0042
19	3	N/90	.0011	4	N/90	.0032	8	N/120	.0042
20¶	4	N	.126	5	N/30	.012	4	N/75	.0035
21¶	6	N	.182	4	N/30	.0098	6	N/80	.0048

Operative case—see chart.

that by using a cell suspension free from serum constituents there would be presented a relation between the percentage of cell suspension, chemical percentage, and time index which agrees, at least more nearly, with the law of multiple proportions?

Table 4 gives the chemical equivalents in percentage of a fifteen-minute hemolytic system for a 1% blood cell suspension for different specimens of the same species as designated.

Table 5 gives the equivalent chemical percentages for the fifteen-minute hemolytic systems in the species tested.

The fifteen-minute hemolytic system and its chemical equivalents for the various species having been considered, the next point for dis-

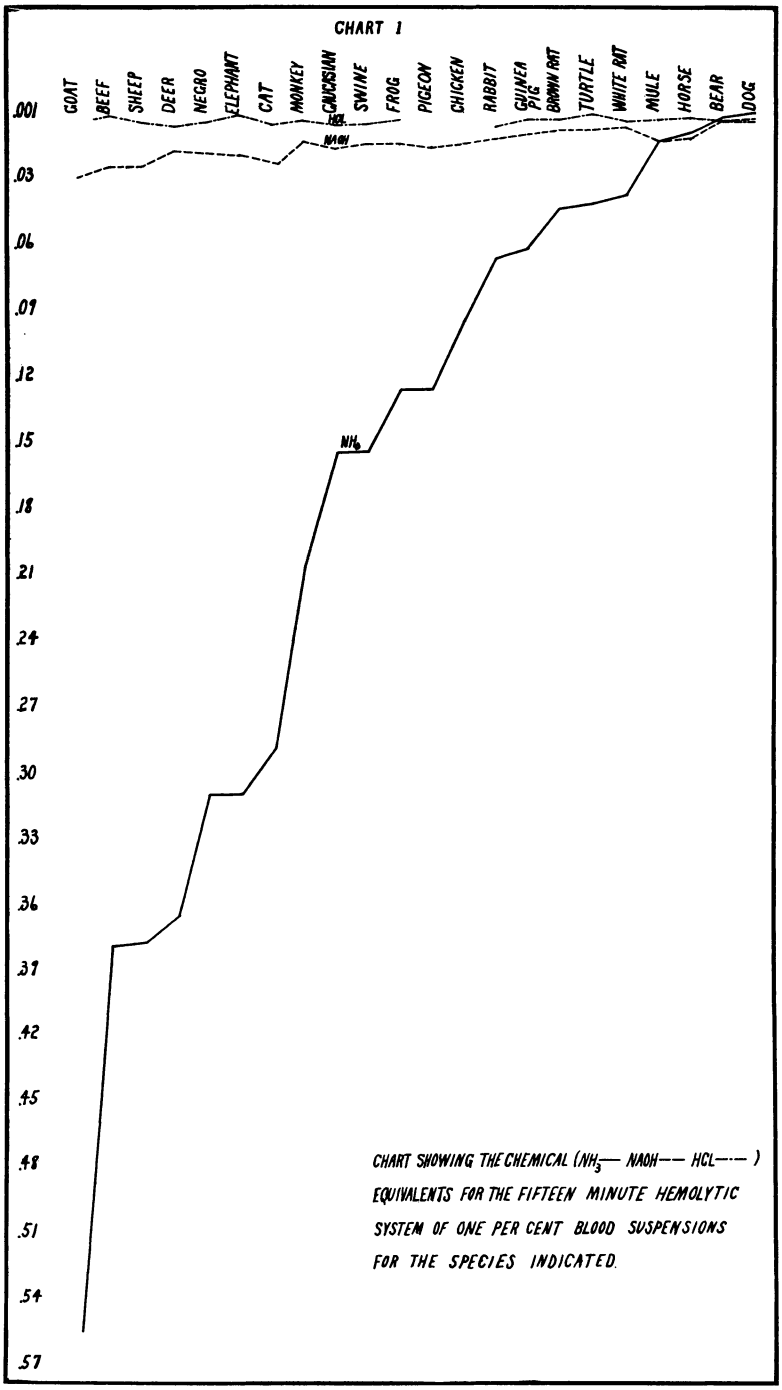
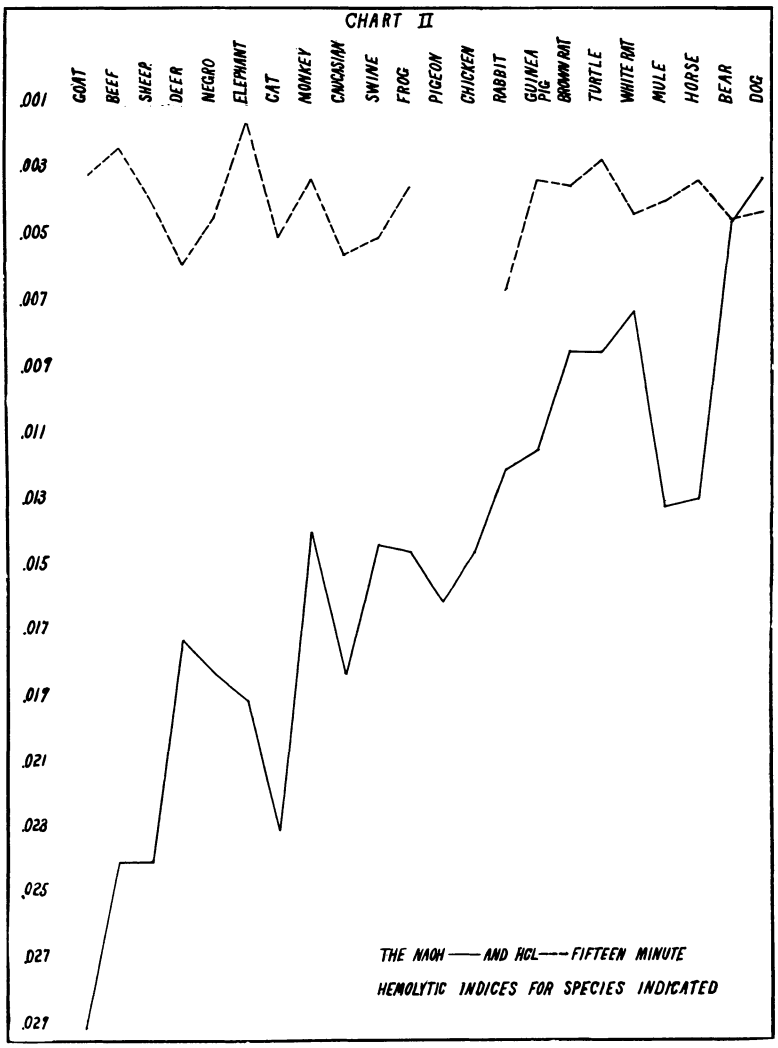


CHART 1
GOAT
BEEF
SHEEP
DEER
NEGRO
ELEPHANT
CAT
MONKEY
CAUCASIAN
HCL
NAOH
SWINE
FROG
PIGEON
CHICKEN
RABBIT
GUINEA PIG
BROWN RAT
TURTLE
WHITE RAT
MULE
HORSE
BEAR
DOG
.001
.03
.06
.09
.12
.15
.18
.21
.24
.27
.30
.33
.36
.37
.42
.45
.48
.51
.54
.57
NH3
CHART SHOWING THE CHEMICAL (NH3— NAOH-- HCL---) EQUIVALENTS FOR THE FIFTEEN MINUTE HEMOLYTIC SYSTEM OF ONE PER CENT BLOOD SUSPENSIONS FOR THE SPECIES INDICATED.

cussion would seem to be the time indices—whatever they chance to be from 3 to 140 minutes—of the chemical hemolysin for the various species. The tabulations of the time indices of the blood of the horse



(6 specimens, Tables 6 and 7) and of the monkey (3 specimens, Tables 8, 9, and 10) are here presented in order to illustrate the degree of variation between different specimens of the same species.

TABLE 5

SUMMARY OF THE TABULATIONS IN TABLE 4

Species	NH_3 %	NaOH %	HCl %
Goat	.548	.029	.0032
Beef	.378	.024	.0023
Sheep	.377	.024	.0039
Deer (Virginia White Tail)	.364	.0172	.0058
Negro	.308	.0182	.0044
Elephant (Indian)	.308	.019	.0015
Cat	.288	.023	.005
Monkey (Rhesus)	.207	.0139	.0033
Caucasian	.154	.0162	.0055
Swine	.154	.0143	.005
Frog	.126	.0145	.0035
Pigeon	.126	.016	.....
Chicken	.094	.0145	.....
Rabbit	.066	.012	.0066
Guinea-pig	.0629	.0104	.0033
Brown rat	.045	.0084	.0035
Turtle	.043	.0084	.0027 Incomplete
White rat	.038	.0072	.0043
Mule	.013	.0131	.0039
Horse	.009	.0129	.0033
Bear (American Brown)	.0025	.0045	.0044
Dog	.0011	.0032	.0042

TABLE 6

THE TIME INDICES AND THEIR CORRESPONDING NH_3 PERCENTAGES FOR 6 SPECIMENS OF 1% DEFIBRINATED HORSE BLOOD IN 0.9% NaCl SOLUTION

NH_3 %	Specimen A Time in Minutes	NH_3 %	Specimen B Time in Minutes	Specimen C Time in Minutes	Specimen D Time in Minutes	Specimen E Time in Minutes	Specimen F Time in Minutes
.0033	55	.0022	..	82	85	80	..
.00654	25	.0043	45	38	32	40	35
.00964	15	.0064	30	27	22	28	17
.0126	12	.0084	20	18	15	17	14
.0154	10	.0106	15	15	10	15	12
.0182	8	.012	14	13	8	11	10
.0207	7	.013	12	11	7	9	8
.0236	6	.015	12	10	..	8	7
.0268	5	.017	11	9	6	..	..
.0285	4	.018	9	8	..	..	6
.0308	4	.02	8	8	..	..	..
.0326	4	.022	7	7	5	6	5

In consideration of the high dilution of the chemical hemolysin it is to be noted that there is but slight variation in the time indices for both the lowest and the highest percentages. Attention is called to the fact that the fifteen-minute hemolytic system occurs in the third pair with specimen A, and in the fourth and fifth in the remaining specimens. In considering the fifteen-minute system of C and D it should be borne in mind that there is only 0.0022% difference in the chemical hemolysin contained in each system. Yet, even in these small percentages, there is but slight variation in the time indices for the

six specimens of horse blood; the conditions being similar for dog's blood—the requirement of small quantities of the hemolysin—it is found that this uniformity also holds true. It requires close observation, however, to determine the end point of hemolysis with NH_3, as it is not so well defined as in the case of NaOH.

TABLE 7

THE NaOH PERCENTAGES AND THEIR TIME INDICES FOR 1% HORSE BLOOD

NaOH %	Specimen A Time in Minutes	Specimen B Time in Minutes	Specimen C Time in Minutes	Specimen D Time in Minutes	Specimen E Time in Minutes	Specimen F Time in Minutes
.0028	..	..	..	..	..	..
.0055	..	..	80	85	..	..
.0081	40	38	39	40	38	30
.0106	19	20	20	20	21	17
.0131	14	15	14	15	16	13
.0155	12	11	11	11	11	10
.0173	10	9	..	10	..	..
.0198	8	8	9	..	..	..
.0221	7	7	6	6	..	..
.024	6	6	..	..	..	..
.0261	6	..	..	..	..	..
.028	5	..	6	5	6	5

The slight variation in the time indices in Table 7 may be explained, for the most part, by errors in measurement. The end-point of hemolysis with NaOH is well defined with all species, and the similarity of the time indices with this chemical is a notable feature.

TABLE 8

THE HCl PERCENTAGES AND THE TIME INDICES FOR 1% HORSE BLOOD

HCl %	Specimen A Time in Minutes	Specimen B Time in Minutes	Specimen C Time in Minutes	Specimen D Time in Minutes	Specimen E Time in Minutes	Specimen F Time in Minutes
.0027	..	..	..	..	..	..
.0033	17	15	17	19	17	12
.0039	8	8	8	8	6	5
.0044	7	7	6	6	4	4
.005	5	5	4	4	..	..
.0055	4	4	..	..	..	..
.006	4	4	4	4	3	3
.0065	..	..	..	..	..	..
.007	..	..	..	..	..	..

The hemolysis of horse blood with HCl (Table 8) breaks off abruptly at 0.0033%, and it is found that even with an exposure of several hours no hemolysis appears in the 0.0027% specimens. Reference to the HCl hemolytic diagram shows that the indices do not extend over a period of more than 15 to 30 minutes. This short index

is probably due to the acid neutralization of the alkalinity of the blood serum, with the formation of neutral salts; the latter prevents hemolysis in some instances much longer than does 0.9% NaCl solution.

TABLE 9

THE TIME INDICES AND THEIR CHEMICAL EQUIVALENTS FOR 1% MONKEY BLOOD IN 0.9% NaCl. THE SIMILARITY IN TIME INDICES FOR DIFFERENT SPECIMENS IS IMPORTANT

NH_3 %	Specimen A Time in Minutes	Specimen B Time in Minutes	Specimen C Time in Minutes
.033	..	54	52
.0654	40	49	38
.0964	30	30	30
.126	27	26	26
.154	22	20	21
.182	18	17	18
.207	15	15	16
.236	13	13	14
.261	12	12	12
.283	10	11	10
.208	9	10	9
.326	8	9	9

TABLE 10

THE TIME INDICES AND THEIR CHEMICAL EQUIVALENTS FOR 1% MONKEY BLOOD IN 0.9% NaOH

NaOH %	Specimen A Time in Minutes	Specimen B Time in Minutes	Specimen C Time in Minutes
.0022	..	..	..
.0043	..	..	..
.0064	56	60	60
.0084	35	35	36
.0104	26	26	26
.0122	20	20	20
.0137	15	15	15
.0157	13	13	14
.0175	12	11	12
.019	10	10	11
.0208	9	9	10
.0221	8	8	9

TABLE 11

THE TIME INDICES AND THEIR CHEMICAL EQUIVALENTS FOR 1% MONKEY BLOOD IN 0.9% HCl

HCl %	Specimen A Time in Minutes	Specimen B Time in Minutes	Specimen C Time in Minutes
.002	50	..	..
.0027	28	30	27
.0033	14	19	15
.0039	10	14	10
.0044	7	10	..
.005	6	..	4
.0055	5	5	..
.006	5	..	..
.0065	4	..	..
.007	4	4	3

CONSIDERATION OF CHART III

In Chart III the species tabulated, according to their hemolytic time indices would seem to fall into 4 groupings: Group 1, dog, bear, and horse, reacting with the smallest percentage of NH_3; Group 2, the rodents, and in addition the mule and the turtle, requiring higher percentages of NH_3; Group 3, pigeon, frog, Caucasian, swine, and monkey, next in order as to NH_3 requirement; Group 4, chiefly of herbivora—elephant, cow, deer, sheep, and goat—and with these the negro, chicken, and cat, requiring the highest percentages of NH_3.

It may be interesting to note that there appears to be an appreciable difference between the time index of the horse and that of the mule as determined by the NH_3 hemolysis. Altho these two species have not as yet been differentiated by the biologic tests (precipitin, complement fixation, and specific hemolysins), yet there is for the same time-exposure a definite chemical index for the eight horses as differentiated from that of the four mules presented in this work. The percentage of NH_3, corresponding to the fifteen-minute hemolytic system, for the mule (0.033%) is 3 times greater than that for the horse (0.01%).

There is also a difference between the Caucasian and the negro time indices, as appears in this work with 10 Caucasian specimens and 4 negro specimens. Of the four negroes tested, 2 were full-blood as nearly as could be determined; while the other two presented color which would indicate about one-fourth negro. Unless exhaustive work may demonstrate whether or not the time indices will show proportionally the varying dilutions of admixture of Caucasian and negro blood it would be unwise more than to notice the distinct variation found between the blood of the eight Caucasians and the four negroes here reported.

The NH_3 percentage requirements of the fifteen-minute hemolytic system for the two extreme indices (dog and goat) represent a considerable difference—the percentage for the goat being 504.5 times greater than that for the dog. The possibility is apparent of differentiating by the NH_3 index between species in the different groups. The work, however, has not been sufficiently developed to state positively that animals in the same group, giving different indices, can be identified by these indices alone. It is to be noted that the frog and the pigeon, the Caucasian and swine, and the negro and the elephant give the same NH_3 percentages for the fifteen-minute hemolytic system, but

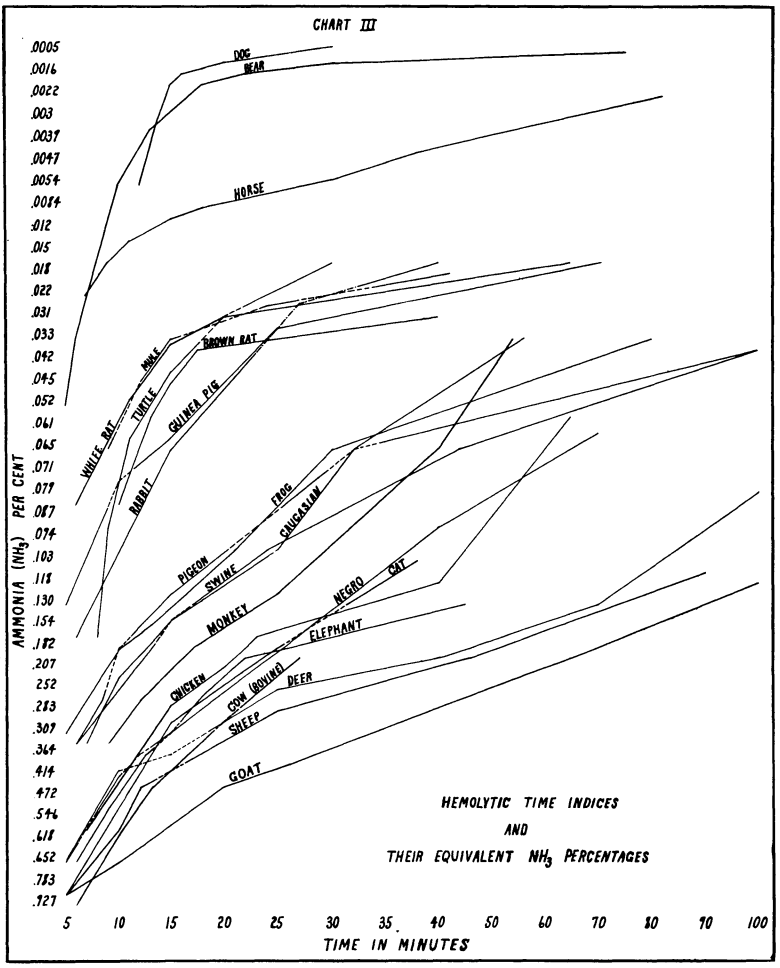
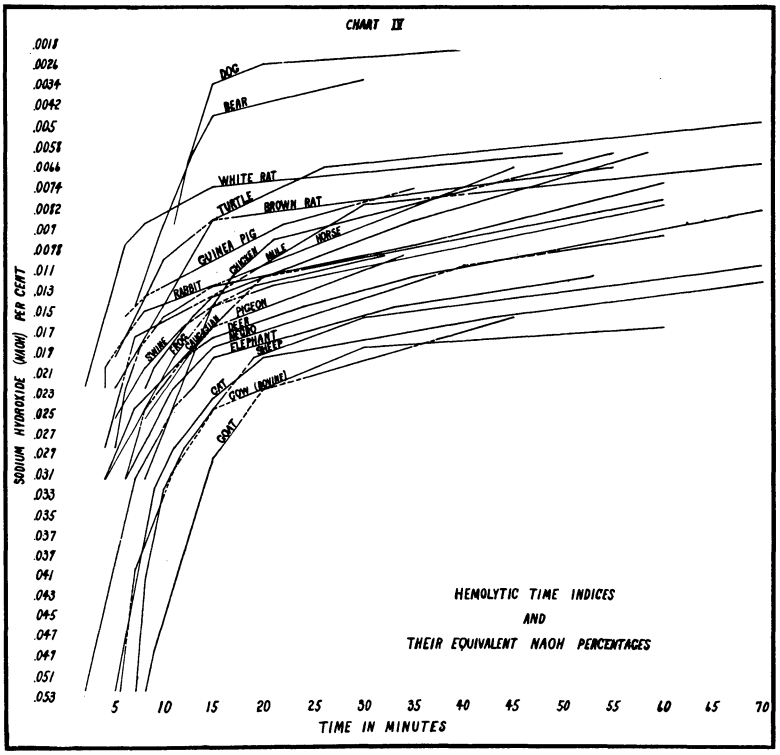


CHART III
AMMONIA (NH_3) PER CENT
.0005
.0016
.0022
.003
.0037
.0047
.0054
.0084
.012
.015
.018
.022
.031
.033
.042
.045
.052
.061
.065
.071
.077
.087
.094
.103
.118
.130
.154
.182
.207
252
.283
.307
.364
.414
472
.544
.618
.652
.783
.927
DOG
BEAR
HORSE
BROWN RAT
MOUSE
WHITE RAT
TURTLE
GUINEA PIG
RABBIT
FROG
CAUCASIAN
PIGEON
SWINE
MONKEY
NEGRO
CAT
ELEPHANT
CHICKEN
COW (BOVINE)
DEER
SHEEP
GOAT
HEMOLYTIC TIME INDICES
AND
THEIR EQUIVALENT NH_3 PERCENTAGES
5 10 15 20 25 30 35 40 50 60 70 80 90 100
TIME IN MINUTES

no two of these require the same percentages of NaOH or HCl for the fifteen-minute system. So, for purposes of identification the time indices of all three chemicals should be employed.

CONSIDERATION OF CHART IV

In the NaOH chart the separate-group character of the NH_3 chart is practically absent. In both charts, however, the species occupy

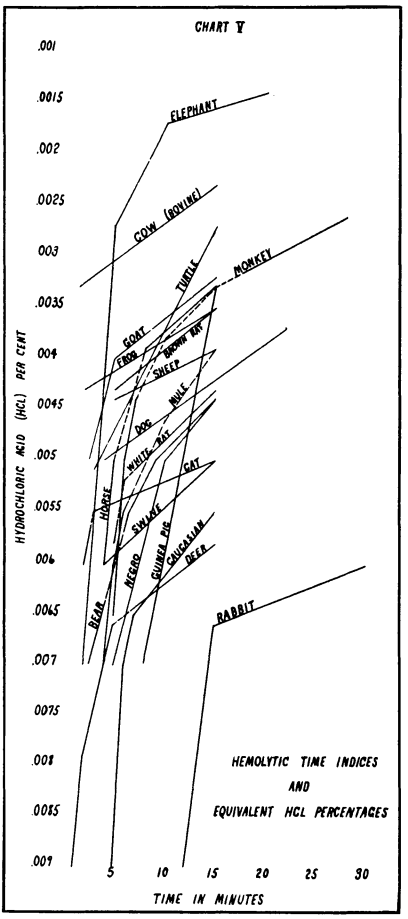


about the same relative positions as to the chemical percentage requirement. In the NaOH chart, as in the NH_3 chart, the dog and the bear react with only traces of the chemical, while the herbivora require the highest percentages.

Attention has been called to the corresponding positions of the NH_3 and NaOH hemolytic time indices in relation to the chemical indices; there would seem to be, however, no special arrangement of

the HCl hemolytic time indices in relation to the alkaline time indices (see Chart V).

A 1% blood suspension from the goat was used for comparing hemolysis by hydrochloric acid with that by sulfuric acid, and hemolysis by sodium, with that by potassium, hydrate. It was found that the time indices were the same for like normalities of the two acids, and also of the two alkalies. For the fifteen-minute system N/1119.6 HCl and H_2SO_4 were required. This normality gives 0.00325% for the HCl and 0.00437% for the H_2SO_4. As the same normalities cause hemolysis in the same time limit, it is probable that the reaction is due to the H ion. Like normalities give the same hemolytic indices; consequently, the percentages of HCl and H_2SO_4 must be to each other as their molecular weights:



$$\frac{3.645}{.00325} = \frac{4.9}{.00437}$$

$$1119.6 = 1119.6$$

For the fifteen-minute hemolytic system N/135 NaOH and KOH were required. This normality gives 0.029% for the NaOH and 0.041% for the KOH. The hemolysis must depend on the hydroxyl (OH) ion, as the acids depend on the H ion. Here again the percentages of NaOH and KOH must be to each other as their molecular weights:

$$\frac{5.6}{.041} = \frac{4.0}{.029}$$

$$135 = 135$$

For the fifteen-minute hemolytic system N/3.66 NH_3 is required, which gives 0.464%. The normality of NH_3 varies considerably from that of the alkali hydroxids, but this variation probably can be explained by the different constitution of the NH_3 molecule when combined with water.

To determine the effect of the blood serum components—protein, acid, alkaline, and neutral salt ions—on the hemolytic index, it is necessary to find the indices for both the unwashed and the washed blood cell suspensions. To show this influence the following experiments with rabbit and mule blood are given:

The unwashed cell suspension was made up in the usual manner (1 c.c. of defibrinated blood to 99 c.c. of 0.9% NaCl), while the washed cell suspension was prepared by washing by centrifugation 1 c.c. of the defibrinated blood in 75 c.c. of 0.9% NaCl solution 3 successive times. The cells were finally taken up in 99 c.c. of 0.9% NaCl solution.

These suspensions were tested with the results shown in Table 12.

TABLE 12

EFFECT OF BLOOD SERUM COMPONENTS ON THE HEMOLYTIC INDEX

Experiment 1			Experiment 2			Experiment 3		
NH_3 %	Unwashed Suspension	Washed Suspension	NaOH %	Unwashed Suspension	Washed Suspension	HCl %	Unwashed Suspension	Washed Suspension
.016	..	20	.0022	..	..	.002	..	..
.0327	24	15	.0043	..	..	.004	..	13
.0432	21	12	.0064	..	25	.0058	17	9
.063	19	9	.0084	30	14	.0077	15	7
.077	17	7	.0104	19	11	.0098	12	6
.091	15	6	.0122	15	6	.0113	9	5
.103	13	5	.0139	13	5	.0127	7	5
.118	12	4	.0157	9	5	.0143	7	4
.130	11	4	.0175	6	4	.016	6	4
.141	10	3	.019	5	3	.0172	6	4
.154	9	2	.0208	..	..	.0185	5	3
.163	4	2	.0221	..	..	.02	5	3

It will be observed in Experiment 1 that for the fifteen-minute hemolytic system the NH_3% requirement is for the unwashed suspension almost 3 times greater (0.091%) than for the washed suspension (0.0327%), the washed cells being hemolyzed more readily, and the serum constituents having an inhibiting power of about 66% of the NH_3. The causes of this inhibition will be noted later in this paper.

In Experiment 2 the difference in the NaOH time indices for the unwashed and the washed cell suspensions is not so great as is the

difference in the corresponding NH_3 time indices. There is for the unwashed suspension a requirement of 0.0122% NaOH for the fifteen-minute system; while 0.0083% is required for the washed suspension. These percentages represent a difference of 0.0038% between the two suspensions with NaOH; whereas the difference is 0.0573% between these two suspensions with NH_3.

Another method of reaching the same conclusion in considering the combining power of the serum, or the inhibiting power of its acid or salt ionic content, is to increase the NaOH% in the unwashed series until it is equal to the difference in percentage for the fifteen-minute system in the two suspensions.

Experiment 4 shows the time indices of the unwashed and the washed blood cell suspensions when treated with the two chemical indices (that for the unwashed increased 0.0043%).

TABLE 13
EXPERIMENT 4

NaOH %	Unwashed Suspension	NaOH %	Washed Suspension
.0065	50	.0022	..
.0086	23	.0043	40
.0107	17	.0064	22
.0127	12	.0084	14
.0147	9	.0104	10
.0165	8	.0122	8
.0182	7	.0139	7
.0257	7	.0157	6
.0218	6	.0175	5
.0233	5	.019	4

By increasing the NaOH 0.0043% in the series of unwashed suspensions the time indices of the two suspensions tend to duplicate each other. This result verifies the conclusion in the consideration of Experiment 3; namely, that the combining power of the serum constituents is about 0.0043% NaOH.

Furthermore, if the standard chemical index—from 0.0022% to 0.0221% NaOH—be added to the two suspensions and 0.0043% HCl be added to the washed suspension series, the similarity in the time indices of the two suspensions will again show that the inhibiting power of the serum is equal to about 0.0043% NaOH.

In Experiment 3 it is shown that for a fifteen-minute system 0.0077% HCl is required for the unwashed suspension; while 0.004% is required for the washed suspension. There is then a difference of 0.0037% HCl for the washed and the unwashed suspensions. This is

verified in Experiment 5, in which the acid was increased 0.0042% in the washed series with the result that the time indices were similar for the two suspensions.

This inhibition of hemolysis by serum for the fifteen-minute system to the extent of 0.0042% HCl may be verified a third time by adding its equivalent NaOH% to the washed cell series with the result that the time indices are similar.

It may be stated that the difference in the time indices with the three chemicals varies for different species, and may we not find a sufficient variation for different species to justify the application of this method in a study of the physical chemistry of the blood of different species? The work is not yet sufficiently advanced to state whether or not the inhibiting action of the serum components is due to the power of the protein to combine with the alkali, or to the pro-

TABLE 14

EXPERIMENT 5

HCl %	Unwashed Suspension	HCl %	Washed Suspension
.0062	..	.002	..
.0082	15	.004	13
.01	13	.0058	9
.0119	9	.0077	7
.014	7	.0098	6
.0155	8	.0113	5
.0169	7	.0127	5
.0185	7	.0143	4
.0202	6	.016	4
.0214	5	.0172	3
.0227	5	.0185	3
.0272	4	.02	3

tective film surrounding the cell, or to the action of neutral salt or acid ions. Acid hemolysis may be influenced by any of these factors, together with that of the alkalinity of the serum.

In pursuing this phase of the work it is hoped that by the aid of the red blood cells as an indicator, means may be developed by which the percentage of the serum components can be accurately determined. The work already accomplished in this direction will be reported in a later paper.

The variation in the time indices caused by increasing the NaCl is shown in Experiments 6, 7, and 8 with rabbit blood suspensions.

In Experiment 6 the blood cell suspension in Series A was 1 c.c. defibrinated blood to 99 c.c. 0.9% NaCl solution; whereas, in Series

B, 1 c.c. of the defibrinated blood was made up in 99 c.c. 2.5% NaCl solution. The influence of increasing the NaCl in Series B is noted by comparing the time indices of this series with those of Series A. In Series B the presence of the NaCl accelerated the NH_3 hemolysis in the first two specimens; while the time for the NH_3 hemolysis of the

TABLE 15

EXPERIMENT 6

NH_3 %	Series A 0.9 % NaCl	NH_3 %	Series B 2.5 % NaCl
.033	65	.033	25
.0654	20	.0654	19
.0964	15	.0964	20
.126	12	.126	18
.154	11	.154	14
.182	10	.182	11
.207	9	.207	9
.236	8	.236	8
.261	7	.261	7
.283	6	.283	6

corresponding specimens in the physiologic solution was the usual requirement. It should be noted further that there was a retardation of hemolysis in Specimens 3, 4, 5, and 6 of Series B in comparison with the hemolysis in the corresponding specimens in the physiologic salt solution. There is no difference in the indices of the two series in

TABLE 16

EXPERIMENT 7

NaOH %	Series A 0.9 % NaCl	NaOH %	Series B 2.5 % NaCl
.0031	..	.0031	..
.0061	38	.0061	35
.0093	18	.0093	19
.0118	12	.0118	13
.0145	10	.0145	11
.0172	9	.0172	10
.019	8	.019	9
.022	7	.022	7
.024	6	.024	6
.026	6	.026	6

the remaining specimens. It is important to note that the hyperisotonic salt solution accelerates the hemolysis of certain NH_3 percentages; while with the other percentages of NH_3 there is a retardation.

The increase of the NaCl content influences the NaOH time indices as shown in Experiment 7 (Table 16).

There is but slight variation in the NaOH time indices for the isotonic and the hyperisotonic salt solutions. The acceleration and retardation in hyperisotonic Series B correspond to acceleration and retardation in the NH_3 Series B in Experiment 6.

The effect of hyperisotonicity on the HCl hemolytic time index is shown in Experiment 8 (Table 17).

TABLE 17
EXPERIMENT 8

HCl %	Series A 0.9 % NaCl	HCl %	Series B 2.5 % NaCl
.0014	..	.0014	..
.0028	..	.0028	..
.0041	35	.0041	..
.0054	22	.0054	..
.0066	15	.0066	..
.0079	12	.0079	..
.0089	8	.0089	..
.01	7	.01	..
.0112	6	.0112	..
.012	5	.012	..

In Experiment 8 with HCl hemolysis the hyperisotonic (2.5%) condition of the salt solution entirely prevents the diffusion of the hemoglobin. In the last six specimens of Series B, however, there is a change in the appearance of the suspensions. These acquire a yellowish tinge, and the cells have a tendency toward flocculation.

TABLE 18
EXPERIMENT 9

NH_3 %	0.9% NaCl	1.3% NaCl	1.5% NaCl	2.0% NaCl
.0022	..	..	..	..
.0043	40	..	..	60
.0064	26	36	42	50
.0084	19	25	28	35
.01	14	23	24	26
.012	12	21	21	20
.013	11	16	19	24
.015	..	14	18	23
.017	9	..	..	22
.018	..	13	16	..
.02	9	..	..	..
.022	8	12	14	16

The varying degrees of inhibition of NH_3 hemolysis of mule-blood suspension obtained by successively increasing the percentages of NaCl are shown in Experiment 9 (Table 18).

It may be noted in this experiment that with the gradual increase in NaCl percentage there is a relative increase in the time indices.

The inhibition of NaOH hemolysis of mule blood by increasing the NaCl solution to 2% is shown in Experiment 10 (Table 19). For the same hyperisotonic salt solution the retardation of NaOH hemolysis is not so great as of NH_3 hemolysis.

TABLE 19
EXPERIMENT 10

NaOH %	Series A 0.9% NaCl	Series B 2.0% NaCl
.0028	..	..
.0055	..	..
.0081	32	42
.0106	18	20
.0131	15	16
.0155	12	13
.0173	11	11
.0198	..	..
.0221	7	8
.024	7	7

The influence on HCl hemolysis of increasing the NaCl content of the suspension to 1.26% is shown in Experiment 11. The inhibition of this hemolysis by the increased NaCl is not marked; whereas with rabbit blood, as shown in Series B of Experiment 8, the inhibition is complete.

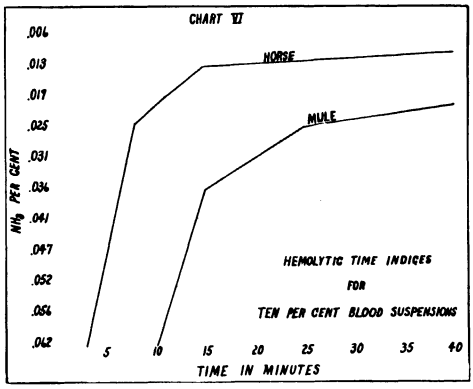
Attention has already been called to the possibility of increasing the difference between the time indices for certain species by increasing the percentages of cell suspensions tested. Reference to the fifteen-minute hemolytic indices of the 1% blood suspension will show

TABLE 20
EXPERIMENT 11

HCl %	Series A 0.9% NaCl	Series B 2.5% NaCl
.0033	..	..
.0039	14	30
.0044	6	6
.005	5	7
.0055	4	7
.006	4	6
.0065	5	5
.007	6	4

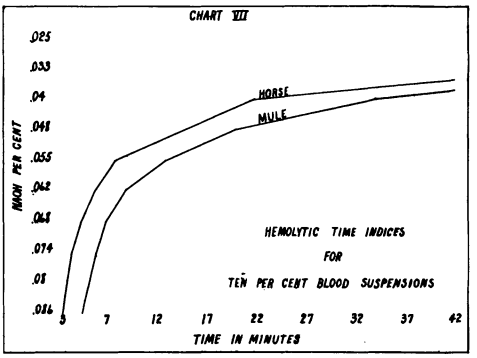
that there was a difference of only 0.004% NH_3 between the horse and the mule. Whereas, for the fifteen-minute system, Chart VI shows for the 10% blood suspension of these two species a difference of 0.023% NH_3. For the 1% blood suspension the NaOH fifteen-minute system shows a difference of 0.001%; while for the 10% blood sus-

pension Chart VII shows for the same time system a difference of 0.004% NaOH. In these two species the NH_3 requirement for the 10% blood suspension was 5.6 times greater than that for the 1% blood suspension; and the NaOH, 4 times greater for the 10% than for the 1% suspension.



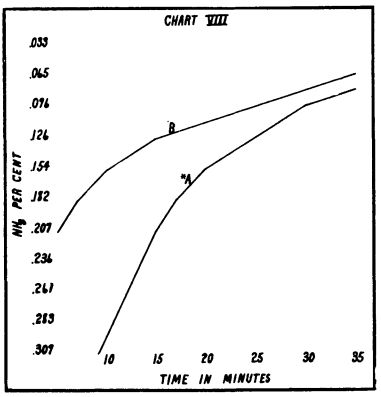
A considerable variation will be noted between the time indices for the normal and for the sick monkey. The greatest variation is in the chemical indices for the eight-minute hemolytic system,—a difference in NH_3 of 0.151%; while for the fifteen-minute system this difference is 0.081%, and as the time increases the indices tend to converge,—at the end of 35 minutes there being a difference of only 0.01% (see Chart VIII).

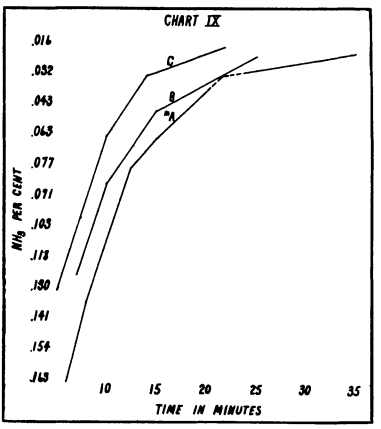
From the experimental viewpoint this decrease in the time index for the sick monkey as compared with that of the normal would seem to be due to a hypo-isotonic condition of the blood. Chart VIII may be compared with Chart X to illustrate the effect of different degrees of isotonicity.

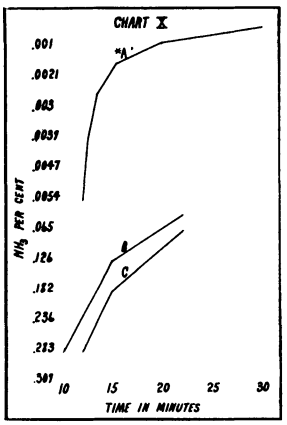


In Chart IX there is a shorter time index for the two sick rabbits than for the normal rabbit. There is a slight, but well-defined, variation between A and B; while C shows a considerable difference from A. This difference may possibly be due to an increase in the alkalinity of the blood, or to a decrease in the neutral salt content or acid ions, or to the combining power of the protein.

In Chart X it is shown that for the fifteen-minute hemolytic system 60 times more NH_3 is required for B (one of the infected dogs) than







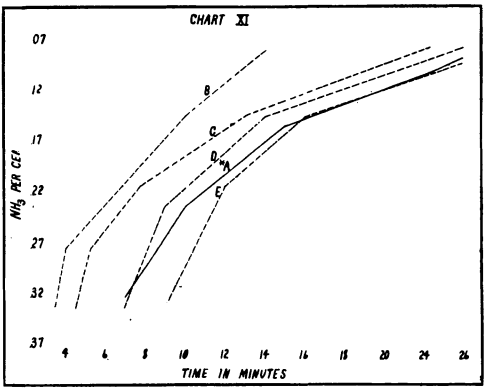


Chart VIII. A = the hemolytic time index with NH_3 for a normal monkey. B = the hemolytic time index with NH_3 for a monkey which had cage paralysis.

Chart IX. A = the hemolytic time index with NH_3 for the normal rabbit. B = the hemolytic time index with NH_3 for a rabbit which was strongly reactive in precipitating beef serum. C = the hemolytic time index with NH_3 for a rabbit infected with the streptococcus of chronic arthritis.

Chart X. A = the hemolytic time index with NH_3 for the normal dog. B and C = the hemolytic time indices with NH_3 for 2 operative dogs in which there was infection following the removal of a section of the intestine.

Chart XI. A = the hemolytic time index and its equivalent NH_3 percentage for normal human blood. B, C, D, and E = the hemolytic time indices and their equivalent NH_3 percentages for 4 cases of scarlet fever. Specimens B and D were obtained from patients during the height of the febrile state; while C and E were taken from patients in a later stage of the disease.

for A (normal dog); while for C (the other infected dog) 86.6 more is required than for A.

As shown in Experiments 6 and 9, an increase in the salt content of 1 of 2 specimens, otherwise identical, retards hemolysis, and, to produce the same time indices for both specimens, it would be necessary to increase the percentage of chemical hemolysin in the salted suspension. It may also be stated that by increasing the acidity, the same result is obtained.

Attention is called to the fact that, the chemical index being the same, the time index for the infected dog would be much longer than for the normal dog. In all other pathologic specimens reported in this paper the reverse of this is true.

SUMMARY AND CONCLUSIONS

For the fifteen-minute hemolytic system there is a marked difference in the chemical requirements (NH_3, NaOH, and HCl) for some species, while the difference is not so marked for others. For this system one of the three chemical hemolysins may be of the same percentage for two species; invariably there appears however, a difference in the percentage requirement for either one or both of the other two chemical hemolysins.

The NH_3 hemolytic time indices divide the animals tested into 4 fairly distinct groupings.

The position of the NaOH hemolytic time indices of the different species corresponds closely to that of the NH_3 indices.

There is no special arrangement of the HCl time indices for the different species with relation to the alkaline indices.

By the use of the chemical and hemolytic time indices blood cell suspensions of different species can be identified with a considerable degree of accuracy.

By increasing the percentage of blood from 1% to 10% a greater variation in the time indices of different species may be found.

Alkaline hemolysis may be considered due to the hydroxyl group, and acid hemolysis due to the H ion.

The hemolysis of the red blood cell may be used as an indicator to determine the degree of acidity or alkalinity of certain solutions.

As an indicator the cells are affected by the isotonicity of the blood suspension.

Hemolysis may be an aid in determining molecular weights.

Alkaline hemolysis can be influenced by acids, and acid hemolysis by alkalies. Both acid and alkaline hemolysis can be influenced by the neutral salt content of the suspension.

In most of the specimens tested there was shown a distinct variation between the normal and the pathologic blood of the same species. The time indices in the majority of the latter specimens were increased or decreased in comparison with the normal.

It is possible that the variation between the normal and the pathologic specimens may be accounted for by increased alkalinity, or decreased acidity, or by the variation in the neutral salt content.